EP333988 (1)

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 4 : C07D 257/00, C07F 9/94 C07F 7/24, 5/06, 3/08 C07F 3/10, 5/00, 3/12 C07F 3/00

A1

(11) International Publication Number: **WO 89/11475**

(43) International Publication Date: 30 November 1989 (30.11.89)

(21) International Application Number: PCT/US89/02201

(22) International Filing Date: 24 May 1989 (24.05.89)

(30) Priority data:
198,538 25 May 1988 (25.05.88) US

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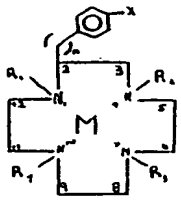
(81) Designated States: AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent).

Published
With international search report.

(54) Title: MACROCYCLIC CHELATES AND METHODS OF USE THEREOF



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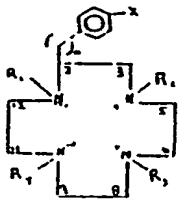

(II)

(57) Abstract

The invention is a chelate having general formula (I), wherein R_{1-4} is $-CH_2COOH$; n is 1 to 5; X is a member selected from the group consisting of $-NO_2$, $-NH_2$, -NCS, $-NHCOCH_2$-Z with Z being a member selected from the group consisting of Br and I, -COOH; $-OCH_2OOCH$; and M is a metal ion being a member selected from the group of elements consisting of Bi, Pb, Y, Cd, Hg, Al, Th, Sr, and Lanthanides. The invention can include a chelate wherein M is a copper ion and n is an integer from 2 to 5. The invention includes chelate conjugates of general formula (I) and ligand conjugates of general formula (II). The invention also includes methods to use these compounds for treatment of cellular disorders and for diagnostic tests.

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MACROCYCLIC CHELATES AND METHODS OF USE THEREOF

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to macrocyclic chelates and methods of use thereof. More specifically, this invention relates to 2-substituted 1, 4, 7, 10-Tetraaza cyclododecane -N, N', N'' N'''-tetraacetic acid, and 2-substituted 1, 4, 7, 10-Tetraazacyclododecane, and analog macrocycles and their uses.

2. Description of the Background Art

Macrocycles have been studied for their usefulness as chelates for numerous metal ions that have therapeutic diagnostic, or other uses. A macrocycle of particular usefulness as a chelate is the 1, 4, 7, 10-Tetra-azacyclododecane -N, N, N, N-tetraacetic acid (DOTA). DOTA compounds have been linked to biomolecules to form delivery systems for the chelated metal ion to specific sites within an organism.

U.S. Patent Number 4,678,667 to Meares et al., discloses a macrocyclic bifunctional chelating agent The chelating agents of this disclosure can include DOTA compound that is a Cu(II) chelate. The usefulness of the chelating agent is limited to the effects of the copper metal ion. The synthesis of this disclosure gives low and not always reproducible results.

An earlier U.S. Patent No. U.S. 4,622,420 to Meares et al. disclosed bifunctional chelating agents of the acyclic ligand ethylene diamene N, N' N'', N''' tetraacetic acid (EDTA) useful for binding metals other than copper such as Indium. These compounds are useful for imaging of tumors.

U.S. Patent Number 4,652,519 to Warshawsky et al., discloses bifunctional chelating agents and process for their production. The compounds disclosed in this patent are analogues of EDTA. These compounds are used to chelate metal ions and are linked to haptens to provide specific site selection within an organism. The compounds of this patent are offered to provide an improved substitutent for the EDTA compounds such as those disclosed in the Meares et al. patent discussed above.

U.S. Patent Numbers 4,454,106 and 4,472,509 to Gansow et al., respectively disclose the use of metal chelate conjugated monoclonal antibodies and the specific metal chelate conjugated monoclonal antibodies. These disclosures provide compounds and methods for treating cellular disorders. Radiometal chelate conjugated monoclonal antibodies specific to a target cell are used to deliver alpha, beta, or Auger electron emitting metal ions. These disclosures are not related to DOTA compounds.

The value of having a ligand conjugate to chelate metal ions for therapeutic, diagnostic, or other uses is of commercial importance. This commercial importance is created by the fact that many metal ions have desirable characteristics for these various uses, but the delivery systems for the metal ions lack specificity to target cells or do not adequately bind the metal ions. Examples of the usefulness of specific metal ions are as follows.

The usefulness of radionuclide materials in cancer therapy is disclosed in the article, Kozak et al., "Radionuclide-conjugated monoclonal antibodies: A Synthesis of Immunology, in Organic Chemistry and Nuclear Science" Trends in Biotechnology. 4(10):259-264 (1985). This article discusses the use of antibody conjugates to deliver either alpha or beta radiation. The value of alpha radiation from bismuth-212 in radionuclide therapy is further discussed in the two articles, Kozak et al., "Bismuth-212-labeled anti-Tac monoclonal antibody: Alpha-particle-emitting Radionuclides as Modalities for Radioimmunotherapy" Proc. Natl. Acad. Sci. U.S.A. 83:474-478 (1986) and Gansow et al., "Generator-produced Bi-212 Chelated to Chemically Modified Monoclonal Antibody for Use in Radiotherapy" Am. Chem. So. Symposium Series 15:215-227 (1984).

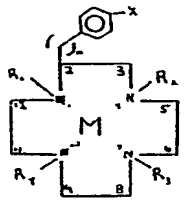
Examples of other uses for chelated metal ions are disclosed in the following articles. Magerstadt et al., "Gd(DOTA): An Alternative to Gd(DPTA) as a $T_{1,2}$ Relaxation Agent for NMR Imaging or Spectroscopy" Magnetic Resonance in Medicine 3:808-812 (1986), discloses the usefulness of gadolinium as a relaxation agent for NMR imaging. The article, Spirlet et al., "Structural Characterization of a Terbium(III) Complex with 1, 4, 8, 11-Tetraazacyclotetradecane- 1, 4, 8,

11-tetraacetic Acid. Lanthanide Ions and the Conformation of the 14-Membered Macrocyles" Inorganic Chemistry 23(25):4278-4283 (1984), discloses the usefulness of lanthanide chelates.

The industry is lacking a DOTA chelate that can be efficiently produced in high yields and that has desirable chelating qualities for numerous metal ions.

SUMMARY OF THE INVENTION

The invention is a chelate having a general formula I:

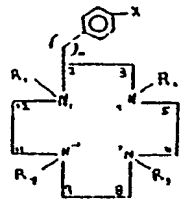


wherein R_{1-4} is $-CH_2COOH$;

n is 1 to 5;

X is a member selected from the group consisting of
$-NO_2$,
$-NH_2$,
-NCS,
$-NHCOCH_2$ - Z with Z being a member selected from the group consisting of Br and I
-COOH;
$-OCH_2COOH$
and M is a metal ion being a member selected from the group of elements consisting of
Bi, Pb, Y, Cd, Hg, Al, Th, Sr, and Lanthanides.

The invention can include a chelate wherein M is a copper ion and n is an integer from 2 to 5. The invention includes chelate conjugates of general formula I and ligand conjugates of general formula II:



The invention also includes methods to use these compounds for treatment of cellular disorders and for diagnostic tests.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates a chemical pathway to produce the preferred embodiment of the invention.

DETAILED DESCRIPTION OF THE INVENTION

The compound of this invention is a substituted DOTA represented by the general formula I shown above or specifically by compound X of Figure 1. Compound X can subsequently be converted to other substituted DOTA compounds, but compound X is the parent compound for such other compounds. The general formula is a 12 membered ring tetraaza macromolecule with the nitrogens in the 1, 4, 7, and 10 positions. Each of the nitrogens is "ribbed" by an ethylene group.

The substituted DOTA ligand represented by compound X of Figure 1 complexes metals. Metal complexes are formed by placing the DOTA into solution with an appropriate metal salt having the metal to be chelated. Metal salts

have to be selected so as to prevent the hydrolysis of the metal. Also, reaction conditions in an aqueous medium have to be chosen such that the metal it not hydrolyzed. For example, a lead nitrate complex, bismuth iodide complex, or yttrium acetate salts can be used to form a metal chelate with lead, bismuth, or yttrium, respectively. General examples of suitable salts include any soluble divalent metal complex or any trivalent metal complex that is not hydrolyzed at pH 4 or below. Thorium requires the use of iodide salt, specifically. The most desirable metal ions for chelation with general formula 1 are members from the group consisting of bismuth, lead, yttrium, cadmium, mercury, actinium, thorium, strontium, and any of the elements of the lanthanide elements. The most desirable elements of the lanthanide series are gadolinium, for use in NMR imaging and as a relaxation agent in NMR imaging, and terbium and europium because of their use as chromophores in time resolved fluorescence spectroscopy. These fluorescent compounds can be useful in an in vitro diagnostic assay where a florescent assay is used rather than a radioactive amino assay.

The X substituent of general formula is desirably a substituent that conjugates the compound with haptans. This substituent is desirably a free-end nitro group which can be reduced to an amine. The amine can then be activated with a compound such as thionyl chloride to form a reactive chemical group such as an isothiocyanate. An isothiocyanate is preferred because it links directly to amino residues of a haptan such as a monoclonal antibody. The amiline group can be linked to an oxidized carbohydrate on the protein and, subsequently, the linkaged fixed by reduction with cyanoborohydride. The amino group can then also be reacted with bromoacetyl chloride or iodoacetyl chloride

to form -NHCOCH$_2$Z with Z being bromide or iodide. This group reacts with any available amine or sulfhydryl group on a hapten to form a stable covalent bond. If tyrosine is used in the formulation of the macromolecule a carboxcylic acid or methoxy carboxylate group can be in this position of the compound. The most desirable substituents for this position are members selected from the group consisting of -NO$_2$, -NH$_2$, -NCS, -COOH, -OCH$_2$COOH, -OCH$_2$COOH and -NHCOCH$_2$-Z with Z being a member selected from the group consisting of bromide and iodide. The preferred substituent for this position is -NCS.

The haptens suitable for linking with the substituent at the X position of general formula I can vary widely. The most desirable haptens are members selected from the group consisting of hormones, steriods, enzymes, and proteins. These haptens are desirable because of their site specificity to tumors and/or various organs of the body. The preferred hapten for use in treating cellular disorders or various disease conditions is a monoclonal antibody.

The compound of this invention can have n equal an integer from 1 to 5. In the preferred embodiment, M equals 2. It is desirable for n to equal 2 versus 1 because the chelating ligand is further separated from the antibody and has more rotation. The increased free rotation allows a metal to chelate with the macromolecule more easily. When n is 3 or greater, the synthesis of the compound becomes lengthy.

Figure 1 illustrates the preferred reaction pathway or process for forming the compound of this invention. This reaction results in a compound of general formula I

wherein n is 1. If n is to equal 2, an additional methylene group would be present between the alpha amino carbon and the aromatic group. This compound is 2-amino-4-nitrophenylbutyric acid.

The process for synthesizing a compound according to this invention first provides a triamine with a substituent is in the 2-position. The embodiment of Figure 1 has a methylene [n = 1] as the initial substituent for linkage. The preferred embodiment has a phenyleythylene group. The process then provides a tetraaza macromolecule having the substituent in the 2 position. Alkylation with bromoacetic acid to form the four carbon to nitrogen bonds of the carboxymethylene substituents at the R1, R2, R3, and R4 in the general formula.

The process of Figure 1 reacts p-nitrophenyl alanine with methanol and hydrochloric acid to form the ester compound II. This ester is reacted with ethylenediamine in the presence of triethylamine to remove the hydrochloride salt of the ester formed in compound II. The condensate of the amide of the ethylenediamine adduct or compound III is subsequently reacted with a diactive ester or compound VI to form a cyclic product or compound VII.

The desired diactive ester VI is formed sequentially from amidodiacetic acid for IV of Figure 1. The amine is first blocked by using the reagent BOC-ON or any other suitable blocking agent, such as FMOC, in the presence of triethylamine which serves to deprotonate the starting material. The subsequent nitrogen blocked diacetic acid V or other such nitrogen blocked compound is then coupled to N-hydroxysuccinimide, or any other suitable compound such as phenols, or N-hydroxydicarboximides which forms a

reactive ester. The choice of compounds which form active esters or blocking groups is within the scope of the art. The coupling is done by dicyclohexyl-carbodiimide or "DCC". This step produces the nitrogen blocked active ester or compound VI.

Ring formation under high dilution conditions between amino acidamide or compound III with the nitrogen blocked active ester of compound VI then occurs. This condensing step forms the triamide macrocycle or compound VII. Compound VII is produced in very high yield. The yield is typically at least about 80 percent. The yield more desirably is between about 80 percent to about 95 percent.

The synthesis of the macrocycle of compound VII may be accomplished by two pathways. Th amine nitrogen of compound VII is deblocked with trifluoroacetic acid or "TFA". This forms the TFA salt of the triamide macrocycle or compound VIII. This compound is reduced with borane/petrahydrofuran or THF. The resulting borane adduct is cleaved by hydrochloric acid to form the substituted tetraazamacrocycle of compound IX. This tetraazamacrocycle can then be alkylated with haloacetic acid in the presence of base to form a nitrobenzyl DOTA or compound X. Alternatively, compound VII can be reduced with borane/THF and reacted with hydrochloric acid to form compound IX directly. This alternative pathway produces slightly poorer yields.

The nitro group of compound X can be reduced with hydrogen over platinum on a carbon catalyst to produce the amino group or the aminobenzyl DOTA depicted as

compound XI. Compound XI can then be reacted with thiophosgene to produce the isothiocyanate or compound XII.

The methodology in column 3 of U.S. Patent Number 4,652,519 to Warshawsky et al., hereby incorporated by reference, provides the methodology to produce the -COOH substituent. This procedure produces the ethylene diamine intermediate. The desired intermediate macrocycle is produced by forming the analogous diactive ester of compound VI by using N, N'-ethylenediaminediacetic acid. Condensation of the diamine with the dinitrogen, diBOC diactive ester produces diamide intermediary which is reduced by diborane to produce the appropriate tetraaza macrocycle. The DOTA ligand can be made from this macrocycle. The synthesis of the X and Z groups are also disclosed by the Warshawsky patent.

The reaction steps described above to produce compounds X, XI, and XII are known. The novel feature of the process of Figure 1 is the cyclization procedure. The conversion reaction of compound IV with compound VI to form the macrocycle and the full reduction of the macrocycle to produce compound X produces the unexpected results of very high yields compound X.

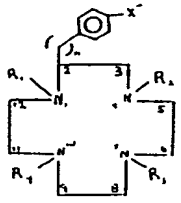
In its preferred embodiment the coupling of an isothiocyanate chelate of compound XII of Figure 1 is done by direct conjugation of the isothiocyanate with a free amino group found in many residues of proteins, enzymes or other compounds such as certain hormones. An example of this situation with a hormone is found with the free amino group provided by the epsilon amino group of the lysine or the terminal amino group as the hormone

peptide chain. Any free amino group can react with the isothiocyanate to form a thiourea linkage which is covalently coupled and irreversible. The use of a steriod as a hapten requires that an amino function be present in the steroid.

An advantage of the amine derivative chelate of compound XI of Figure 1 is that, when coupling to proteins and, in particular, when coupling to antibodies, the carbohydrate of the antibody can be oxidized prior to the coupling reaction. The amine reacts with the aldehyde that is formed on the protein. This aldimine formed can be reduced by cyanoborohydride to form a covalent secondary amine linkage to the antibody in a position that is site specific. This position is away from the binding site of the FAB'2 part of the monoclonal antibody.

A desirable embodiment of the invention is one having copper metal ion and n is an integer from 2 to 5. This embodiment of the invention can be used to label a monoclonal antibody with Cu^{67}. When n is an integer from 2 to 5, there is less hindrance of the chain of the ligand with the protein than occurs when n is 1. When n is an integer from 2 to 5, sufficient space is provided between the ligand and the protein to allow freer rotation of the ligand. This results in more efficient chelation of the copper ion by the resulting conjugate.

An embodiment of the invention involves a ligand-hapten conjugate of general formula II:



This conjugate chelates metal ions. It is desirable to expose many metals to the protein conjugate in a concentrated metal solution for as short a period of time as possible. Certain metals, such as divalent metal ions, react rapidly and directly with the conjugate. The kinetics of the formation reactions for these compounds are so rapid that it is desirable to have the ligand-hapten conjugate available in the pharmacy immediately prior to use. The conjugate can then be mixed in the radionuclide to form a complex and, subsequently, the metal chelate conjugate formed can be purified by, for example, size exclusion high pressure liquid chromatography. A desirable hapten for the ligand conjugate can be selected from the group consisting of hormones, steroids, enzymes, and proteins.

The most commercially useful embodiments of the invention are chelate conjugates having general formula I wherein (1) n is an integer from 1 to 5, (2) X' is a member selected from the group consisting of -NHQ, -NCS-Q, $-NHCOCH_2-Q$, $-OCH_2COOQ$, and -COO-Q with Q being a hapten selected from the group consisting of hormones, steroids, enzymes, and proteins, and (3) M is a metal ion being a member selected from the group of elements consisting of Bi, Pb, Y, Cd, Hg, Ac, Th, Sr, and Lanthanides. These chelates conjugates can deliver radioactive metal ions such as Pb^{212}, Bi^{212}, Y^{90}, Th^{224}, and Sr^{90} to specific cellular disorders.

The preferred embodiment of the invention uses a chelate conjugate binding Pb^{212}. Pb^{212} is a very desirable pharmaceutical compound for delivering both beta and alpha radiation to a selected site for treatment of the cellular disorders. The delivery is made through

the Pb^{212} ion which converts with a 10 1/2 hour half-life into Bi^{212}. Bi^{212} and daughters deliver one alpha particle per Pb^{212} nucleus. The desirable result of this chelate conjugate is that the Pb^{212} half-life is sufficient to allow site selection from the body fluid by the hapten before the alpha particle is emitted.

The invention includes a process for treating cellular disorders. This process uses the chelate conjugate with a hapten having a selective binding site at the cellular disorder. For example, Q can be a monoclonal antibody wherein the antibody is directed and created against an epitope found specifically on the tumor cells. Thus, when Pb^{212} is transported to the antegen site and, subsequently, decays in secular equillibrium to Bi^{212} and its daughters, a beta irradiation is produced from the lead disintegration. A beta radiation is produced by the bismuth daughters. This beta radiation is similar to the beta radiation from Y^{90}, but in addition, each disintegration of bismuth also produces an alpha particle. In this manner, a radiotherapy is provided with a radiation dose from both an alpha and a beta particle. If desired, only Bi^{212} can be introduced in those cases where the disorder to be treated, such as with leukemic cells, can be easily reached within the 1 hour half-life of Bi^{212}. It is also possible to use this method to treat cancers where the cells are widely differentiated. This might be preferred where only a long range beta emitter, such as Y^{90}, is desired. In differing environments, <u>in vivo</u>, the Bi^{212} is retained inside the chelate after the beta emission in differing amounts. Most desirably, at least 95 percent of Bi^{212} remains in the chelate. In an acidic medium, such as the stomach, at least about 70 percent of the

Bi^{212} is retained. Retaining at least about 80 or 90 percent, Bi^{212} is also desirable depending on the medium.

The invention includes a process for diagnostic testing. This process uses a chelate conjugate having general formula I wherein M is a member selected from the group consisting of Pb^{203}, Tc^{99m}, In^{111}, Ga^{67}, Ga^{68}, Sc^{43}, Sc^{44}, Fe^{52}, Fe^{54}, Fe^{56}, Fe^{57}, Fe^{58}, and Co^{55}. The usefulness of metal ions with both in vitro and in vivo diagnostic procedures is disclosed in U.S. Patent Number 4,454,106, hereby incorporated by reference.

The most desirable embodiment of this diagnostic process uses Pb^{203}. Pb^{203} has a 52.1 hour half-life as a gamma emitter. Pb^{203} has a unique property in that it decays at a high percentage only by a single photon emission. This gamma emission is preferred and dominant over all other emissions. This single photon emission makes Pb^{203} useful for single photon emission computed spectroscopy [SPECT] which is a diagnostic tool. Thus, when Pb^{203} is linked by use of the chelate to a hapten which specifically localizes in a tumor, then that particular localization can be three dimensionally mapped for diagnostic purposes in vivo by single photon emission tomography. Alternatively, the emission can be used in vitro in radioimmunoassays.

EXAMPLE 1

The procedures and reagents described above for the preferred embodiment of making the compounds are used for this example.

The antibody specific for the IL-2 antigen is the monoclonal antibody alpha-Tac. This antibody is labelled with the chelate of compound XII of Figure 1 as follows. The antibody is suspended in a buffered normal saline solution having a pH of about 8.5. Solid ligand or compound XII is added to the protein suspension. The protein conjugate forms during reaction overnight and is purified by dialysis against metal-free 0.05 molar citrate/0.15 molar sodium chloride buffer at pH 5.5. Before labelling with metal, the protein is dialyzed against a solution comprising 0.02 molar N-morpholinoethanesulfonic acid and 0.02 molar acetate at pH 5.9.

The protein in solution is labelled with Y^{90} by reacting with an acetate solution of the isotope followed by passage through a TSK 3000 size exclusion column. This is a high pressure liquid chromotography procedure. The compound is mixed with a pharmaceutical excipient and is used in mammals in a therapeutic amount to treat adult T-cell leukemia in mammals. T-cell leukemia displays the characteristics of having an extraordinarily large amounts of IL-2 receptors located on the tumor cells. The antibody localizes specifically to these tumor cells to deliver its radiation.

EXAMPLES 2 and 3

The procedures and reagents described above for the preferred embodiment of making the compounds are used for these examples. The only difference between Example 1 and Examples 2 and 3 are the use of the antibody B72.3 which binds specifically to a glycoprotein on LS-174T cells. This glycoprotein is also in humans which have colon cancer. The model system of this example is an

athymic mouse which has been implanted with LS-174T cells to develop a tumor on the flank of the animal in the location of where the cells were implanted. The diagnostic method used to visualize the growing tumor involves the following components. The chelate of compound 12 is first coupled to gadolinium or Pb^{203} by mixture of the chelate solution at pH 4 to 5 with gadolinium or Pb^{203} nitrate. This material can be then linked directly to the antibody by mixture to react with the protein and purified according to the method of the previous example.

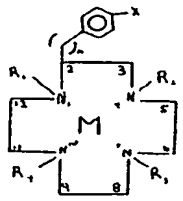
In Example 2, the gadolinium chelate ligand-protein conjugate is injected or introduced into body fluids of a mammal. The gadolinium then localizes along with the antibody to the tumor and conventional resonance magnetic imaging techniques are used to visualize the tumor.

In Example 13, the Pb^{203} is used, the metal labelled protein conjugate is similarly introduced into the mammal, but gamma camera or SPECT imaging is used to visualize the tumor.

WHAT IS CLAIMED IS

1. A chelate comprising:

a general formula I:



wherein R_{1-4} is $-CH_2COOH$;

wherein n is an integer from 1 to 5;

X is a member selected from the group consisting of

$-NO_2$,

$-NH_2$,

-NCS,

$NHCOCH_2$-Z with Z being a member selected from the group consisting of Br and I

$-OCH_2COOH$;

-COOH;

and M is a metal ion being a member selected from the group of elements consisting of Bi, Pb, Y, Cd, Hg, Al, Th, Sr, and Lanthanides.

2. The chelate of claim 1 wherein n is 1 to 2 and X is a member selected from the group consisting of $-NO_2$, -NCS, and $-NHCOCH_2-Z$ with Z being a member selected from the group consisting of Br and I.

3. The chelate of claim 2 wherein M is a member selected from the group consisting of Bi, Pb, Y, Th, Sr, Gd, Eu, and Tb.

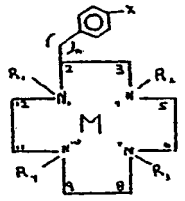
4. The chelate of claim 1 wherein n is 2, X is -NCS, and M is a member selected from the group consisting of Pb212, Pb203, Bi212, Y90, Th224, and Sr90.

5. The chelate of claim 4 wherein M is a member selected from the group consisting of Pb212 and Bi212.

6. The chelate of claim 2 wherein n is 2, X is NCS, and M is a member selected from the group consisting of Eu and Tb.

7. A chelate comprising:

a general formula I:



wherein R_{1-4} is $-CH_2COOH$;

wherein n is an integer from 2 to 5;

X is a member selected from the group consisting of

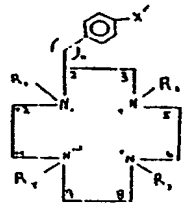
$-NO_2$,
$-NH_2$,
-NCS,
$-NHCOCH_2$ - Z with Z being a member selected from the group consisting of Br and I
-COOH;
$-OCH_2COOH$;
and M is Cu.

8. The chelate of claim 7 wherein n is 2 and X is a member selected from the group consisting of $-NO_2$, -NCS and $-NCOCH_2$-Z with Z being a member selected from the group consisting of Br and I.

9. The chelate of claim 8 wherein X is -NCS.

10. A ligand-hapten conjugate comprising:

a general formula II:



wherein R_{1-4} is $-CH_2COOH$;
n is an integer from 1 to 5;
X' is a member selected from the group consisting of -NH-Q,

$-NCOCH_2$-Q,
$-OCH_2COOQ$, and

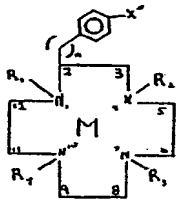
with Q being a hapten selected from the group of hormones, steroids, enzymes, and proteins.

11. The ligand conjugate of claim 10 wherein n is 2 and X' is -NH-C-Q.
‖
S

12. The ligand conjugate of claim 11 wherein Q is a protein, said protein being a monoclonal antibody.

13. A chelate conjugate comprising:

a general formula I:



wherein R_{1-4} is $-CH_2COOH$;
n is an integer from 1 to 5;
X' is a member selected from the group consisting of -NH-Q,
-NHCS-Q,
$-NHCOCH_2-Q$,
$-OCH_2COOQ$, and
O
‖
-C-O-Q
with Q being a hapten selected from the group consisting of hormones, steroids, enzymes, and proteins;

M is a metal ion being a member selected from the group of elements consisting of Bi, Pb, Y, Cd, Hg, Ac, Th, Sr, and Lanthanides.

14. The chelate conjugate of claim 13 wherein n is 1 to 2 and X' is -NH-C-Q.
S

15. The chelate conjugate of claim 14 wherein M is a member selected from the group of Bi, Pb, Y, Th, Sr, Gd, Eu, and Tb.

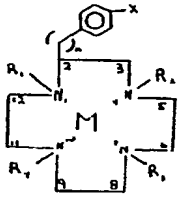
16. The chelate conjugate of claim 15 wherein n is 2 and M is a member selected from the group consisting of Pb^{212}, Pb^{203}, Bi^{212}, Y^{90}, Th^{224}, and Sr^{90}.

17. The chelate conjugate of claim 16 wherein M is a member selected from the group consisting of Pb^{212} and Bi^{212}.

18. The chelate conjugate of claim 15 wherein n is 2 and M is a member selected from the group consisting of Eu and Tb.

19. A process for treating cellular disorders comprising:

introducing into body fluid a solution of metal chelate of a general formula I:



wherein R_{1-4} is $-CH_2COOH$;
n is an integer from 1 to 5;
X is a member selected from the group consisting of -NH-Q,

$-NH-\overset{\overset{S}{\|}}{C}-Q$,

$-NHCOCH_2-Q$,

$-OCH_2COOQ$,

$-\overset{\overset{O}{\|}}{C}-O-Q$

with Q being a hapten selected from the group consisting of hormones, steroids, enzymes, and proteins;

M is a metal ion being a member selected from the group of elements consisting of Bi, Pb, Y, and Gd.

20. The process of claim 19 wherein n is 2, X is $-NH-\overset{\overset{S}{\|}}{C}-Q$, and M is Gd.

21. The process of claim 19 wherein M is Pb212, said Pb212 converts to Bi212 and daughters thereof, said Bi212 emits an alpha particle to treat said cellular disorders.

22. The process of claim 21 wherein n is 2 and X is $-NH-\overset{\overset{S}{\|}}{C}-Q$.

23. The process of claim 22 wherein at least 70 percent of said Bi212 remains in said chelate.

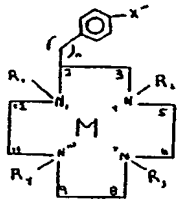
24. The process of claim 23 wherein at least 80 percent of said Bi212 remains in said chelate.

25. The process of claim 24 wherein at least 90 percent of said Bi212 remains in said chelate.

26. The process of claim 25 wherein at least 95 percent of said Bi^{212} remains in said chelate.

27. A process for diagnostic testing comprising:

introducing into a test medium a solution of metal chelate of a general formula I:



wherein R_{1-4} is $-CH_2COOH$;

n is an integer from 1 to 5;

X' is a member selected from the group consisting of -NH-Q,

$-N-\overset{\overset{S}{\|}}{C}-Q$,

$-NHCOCH_2-Q$,

$-OCH_2COOQ$, and

$-\overset{\overset{O}{\|}}{C}-O-Q$ with Q being a hapten selected from the group consisting of hormones, steroids, enzymes, and proteins;

M is a metal ion being a member selected from the group consisting of Pb^{203}, Tc^{99m}, In^{111}, Ga^{67}, Ga^{68}, Sc^{43}, Sc^{44}, Fe^{52}, Fe^{54}, Fe^{56}, Fe^{57}, Fe^{58}, and Co^{55}.

28. The process of claim 27 wherein n is 2, X is -NCS-Q, and Q is a monoclonal antibody.

29. The process of claim 28 wherein M is Pb^{203}.

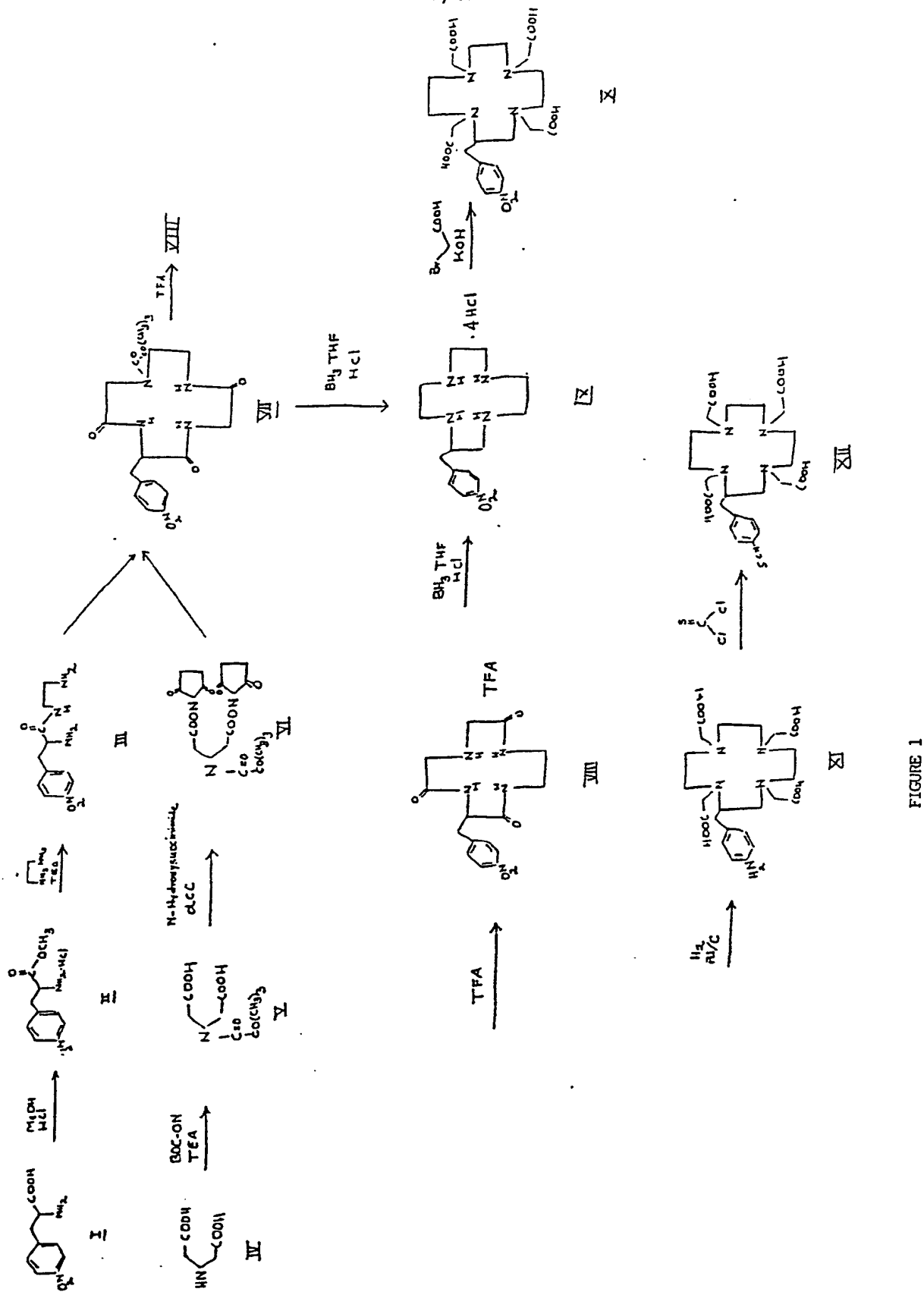


FIGURE 1

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US89/02201

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(4): C07D 257/00; C07F 9/94, 7/24, 5/06, 3/08, 3/10, 5/00, 3/12, 3/00

U.S.Cl.: 540/465, 474; 534/15

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	540/465, 474; 534/15

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

Chemical Abstracts - manual - Vo. 1 (1907)- Vo. 109 (to date)

CA on-line (computer)-1967-1989

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	U.S., A, 3,936,445 (PFITZNER. ET AL) published 3 February 1976. See entire document.	1-9

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
23 AUGUST 1989	01 SEP 1989
International Searching Authority	**Signature of Authorized Officer**
ISA/US	ROBERT C. WHITTENBAUGH

Form PCT/ISA/210 (second sheet) (Rev.11-87)

FURTHER INFORMAT CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers . because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers ... , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

3. ☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

Lack of unity of invention has been found to exist in view of the following independent and distinct inventions. The distinct inventions are as follows:

(I) Chelates in which M is a heavy or light metal embraced by claims 1-9 and classified in 540-465. (See Attachment sheet 1)

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers: 1-9

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

VI. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING (CONTINUED)

(II) Ligand-hapten conjugates and methods for treating cellular disorders embraced by claims 10-26 and classified in 435-188, 530-404, and 436-543.

(III) Methods of diagnostic testing embraced by claims 27-29 and classified in 424-2.

The claims of these 3 groups are directed to different inventions which are not so linked as to form a single general inventive concept. Each group is drawn to an independent and distinct invention.